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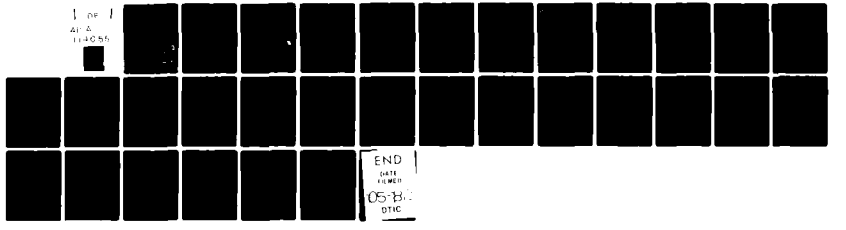

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INSTITUTE REPORT NO. 119

ACUTE ORAL TOXICITY (LD_{50}) OF CHF1 IN RATS

CAROLYN M. LEWIS, MS
MARTHA A. HANES, DVM, CPT VC
and
WILLIAM REIFENRATH, PhD

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT
and
DIVISION OF CUTANEOUS HAZARDS

APRIL 1982

Toxicology Series 24

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Oral Toxicity (LD_{50}) of CHFl in Rats-- Lewis et al
Toxicology Series 24

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

Jack H. Marshall J
(Signature and date)
2 April 1982

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insect repellent

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute oral toxicity potential of CHF1 was determined in rats by using the single dose method. LD1, LD50, and LD95 with their 95% confidence intervals were calculated by probit analysis. The LD50 for male rats was 4632 ul/kg; the LD50 for female rats was 2495 ul/kg. These results place the CHF1 formulation in the slightly toxic range.

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ABSTRACT

The acute oral toxicity potential of CHF1 was determined in rats by using the single dose method. LD_1, LD_{50}, and LD_{95} with their 95% confidence intervals were calculated by probit analysis. The LD_{50} for male rats was 4632 ul/kg; the LD_{50} for female rats was 2495 ul/kg. These results place the CHF1 formulation in the slightly toxic range.

PREFACE

Acute Oral Toxicity GLP Study Report

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

PROJECT: Prevention of Military Disease Hazards 3M16770A871

GLP STUDY NUMBER: 81010

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Martha A. Hanes, DVM, VC

PATHOLOGIST: LTC Paul W. Mellick, DVM, PhD, VC

STATISTICIAN: Virginia L. Gildengorin, PhD, DAC

RAW DATA: A copy of the final report, study protocol, raw data, and standard operating procedure will be retained in the LAIR Archives.

TEST SUBSTANCE: CHF1 - formulation of 50% N,N-diethyl-n-toluamide (m-DEET) in 25% Dow Corning 200 Fluid and 25% isopropyl alcohol.

WORK UNIT: 201 Development of Repellents Against Medically Important Arthropods.

PURPOSE: The purpose of this study was to determine the acute oral toxicity potential of the test substance listed above.

ACKNOWLEDGMENTS

The authors wish to thank SSG Lance White; SP4 Thomas Kellner, BA; SP4 Lawrence Mullen, BS; PFC Evelyn Zimmerman and John Dacey for assistance in performing the research.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 81010 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

John T. Fruin 22Oct81
JOHN T. FRUIN / DATE
LTC, (P), VC
Study Director

William G. Reifenrath 22 Oct 81
WILLIAM G. REIFENRATH / DATE
DAC, Chemist

Paul W. Mellick 22 Oct 81
PAUL W. MELLICK / DATE
LTC, VC
Pathologist

Virginia L. Gildengorin 22 Oct 81
VIRGINIA L. GILDENGORIN, PhD / DATE
DAC, Statistician

Martha A. Hanes 20Oct81
MARTHA A. HANES / DATE
CPT, VC
Principal Investigator

Carolyn M. Lewis 20Oct81
CAROLYN M. LEWIS, MS / DATE
DAC, Data Manager

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

16 Feb 82

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LIAIR GLP study 81010 the following inspections were made:

11 May 81
19 May 81
20 May 81
21 May 81
2 Jun 81

The report and raw data for this study were audited on 30 Oct 81.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1981 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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The goal of the insect repellent program is to develop better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years the Division of Cutaneous Hazards, Letterman Army Institute of Research (LAIR), has tested a large number of chemical compounds, submitted by SRI International, the U.S. Department of Agriculture (USDA), and private industry, against a variety of mosquitoes, sand flies, fleas, bugs, ticks, and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue insect repellent, 71.25% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards has also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

Toxicity Testing Repellent Program

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results that have been obtained in the in vitro and animal tests and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The toxicity tests required for registration of a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic animal toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. An acute oral toxicity (LD_{50}) test is one of the animal toxicity tests for CHF1 requested by the Division of Cutaneous Hazards so that the formulation could be considered for human testing. If adverse toxicity data are obtained with the animal tests, the formulation will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety factor and secondary screen in the repellent development scheme.

Objective of Study

The objective of this study was to determine the acute oral toxicity potential of LAIR formulation CHF1 in rats.

METHODS

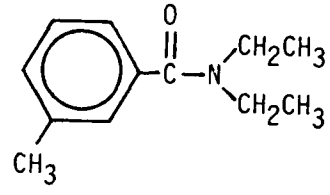
Test Substance

CHF1 is a formulation of 50% N,N-diethyl-n-toluamide (DEET) in 25% Dow Corning 200 Fluid and 25% isopropyl alcohol. The formulation is a suspension that must be agitated to maintain continuity.

1. Chemical Name: N,N-diethyl-n-toluamide

   Chemical Abstract Service Registry No.: 134-62-3

   Molecular structure: $C_{12}H_{17}NO$



   Molecular weight: 191.3 g/moles

   pH: N/A non-aqueous

   Physical state: liquid

   Boiling point range: 288-292 C

   Compound density: 0.996 g/cc

   Compound refractory index: $n_D^{20} = 1.5212$

   Stability: unknown

   Contaminants: contains ortho and para isomers

   Manufacturer: Altrich Chemical Co., Inc., Milwaukee, WI 52301

   Manufacturer Lot No: 032697 (Purity at purchase was 98%, April 1979).

Published Toxicity Data:

Oral LD_{50} (Rat) 2000 mg/kg

Dermal LD_{50} (Rabbit) 3180 mg/kg

Other information:

Listed as an irritant to eyes and mucous membranes, can cause central nervous system disturbances.

2. Chemical Name: Dow Corning 200 Fluid, 1000 cs. viscosity (dimethylsiloxane polymer)

Chemical Abstract Service Registry No.: None

Molecular structure: linear polydimethylsiloxanes

Molecular weight: about 25,000 g/moles

pH: N/A non-aqueous

Physical state: fluid

Compound density: 0.971 g/cc

Compound refractory index: n_D^{20} 1.403

Stability: high thermal stability - manufacturer states unlimited useful life when stored at 25 C.

Purity: unknown

Manufacturer: Dow Corning Corporation, Midland, MI 48640

Manufacturer Lot No: MA 129889

Other information:

Water repellent, low surface tension, low toxicity, essentially non-toxic and non-irritating (although temporary discomfort may result if rubbed into the eye).

3. Chemical Name: isopropanol

Chemical Abstract Service Registry No.: 67-63-0

Molecular structure: $CH_3CHOHCH_3$ (C_3H_8O)

Molecular weight: 60.09 g/moles

pH: N/A non-aqueous

Physical state: clear colorless liquid.

Boiling point: 82.5 C

Compound density: 0.7854 g/cc

Stability: unknown

Purity: unknown

Manufacturer: VWR, Scientific Products,
San Francisco, CA 94119

Quality Control Code: A17

Published Toxicity Data:

Oral LD_{50} (Rat) = 5840 mg/kg

Dermal LD_{50} (Rabbit) = 16,000 mg/kg

Oral LD_{50} (Dog) = 6g/kg

Other information:

Listed as an irritant to eyes acts as a local irritant and in high concentration as a narcotic. It can cause corneal burns and eye damage. Acts much like ethanol in regard to absorption metabolism and elimination but with a stronger narcotic action.

Animal Data

Species: Rat (Rattus rattus)

Strain: Sprague Dawley

Source: Charles River

Sex: Male and Female

Age: 6 weeks at receipt

Method of Randomization: TOXSYS^{R} Animal Allocation Program

Animals in Each Group: 20 animals, 10 males and 10 females

Condition of Animals at Start of Study: Normal

Body Weight Range: 131-198 g at receipt
Males, 204-260 g; Females 156-209 g at dosing

Identification Procedures: Ear tag (SOP-OP-ARG-1)

Pretest Conditioning:

a. Quarantine from 6 - 15 May 1981

b. Animals pre-dosed acclimated with 0.5 cc of water daily from 12 - 15 May 1981.

Justification: The Sprague Dawley rat is a proven sensitive mammalian model for oral LD_{50} determination.

Environmental Conditions

Caging: Number/cage = 1; Type cage used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rodent Diet 5002 ad lib.

Water: Central line to cage battery

Temperature: 19 $\pm$ 3 C

Humidity: 65 $\pm$ 9%

Photoperiod: 0530 - 2000 hr/day (light, 14 1/2 hr).

Dosing

Dr. Reifenrath of Cutaneous Hazards prepared the chemical formulation of CHF1 for the Toxicology Group. Formulation of CHF1 consists of 50% (g/ml) m-DEET, 25% (g/ml) Dow Corning 200 Fluid in isopropanol. Five-hundred milliliters were prepared on 12 May 1981. On dosing day a 2:1 dilution, 100 ml of CHF1 to 50 ml of corn oil, was prepared by CPT Hanes so that all dose groups would be exposed to some level of corn oil and CHF1. Corn oil was selected for its overall acceptable qualities to the animals and because of its solubility in isopropanol. It has been used historically in LD_{50} studies as a carrier for insoluble compounds.

Five dose levels (2000 ul/kg, 2515 ul/kg, 3162 ul/kg, 3976 ul/kg and 5000 ul/kg) were given to both male and female rats (Table 1). The dose for each animal was calculated based on the animals's weight, the dose level desired and the concentration of the dosing solution. The dose was increased by increasing volume rather than the concentration. The volumes ranged from approximately 0.5 ml to 2.0 ml. Vehicle control animals received 2 ml of a mixture of 50% corn oil (g/ml) and 25% Dow Corning 200 Fluid (g/ml) in isopropanol.

All animals were fasted overnight before dosing. All animals received a single dose on 19 May 1981. A 18 gauge, 3 inch gastric lavage needle (Popper and Sons, Inc., New Hyde Park, N.Y.) was used to administer the chemical by gastric intubation. This was performed without sedation or anesthesia of the animals.

Observations

Animals were observed daily during the quarantine period. During the course of the study animals were observed at 0600 and 1800 hours with an alteration on the first and last days. Animals were observed at 1200 and 1800 hours on the first day and at 0600 hours on the day of sacrifice. Findings are reported later in this report.

Statistical Methods

The LD_{50} and slope determination was derived by Bliss probit analysis, as described by Finney (1).

Duration of Study

The actual study lasted 14 days; however, animals were quarantined and acclimated for 12 days before the study began.

Historical Listing of Study Events

6 May 1981	30 male and 31 female rats arrived at LAIR and were housed individually. The animals were ear tagged.
7 May 1981	Animals were weighed. Animals D8100117 and D8100211, randomly selected, were submitted to pathology for quality control.
12 May 1981	Animals were pre-dosed acclimated with 0.5 cc of water.
15 May 1981	Animals were weighed and randomized into dose groups.
18 May 1981	Feed was removed from all cages at 1800 hours.

19 May 1981	Animals were dosed according to groups commencing at 0925 hours.
2 June 1981	Male rats that survived were weighed, sacrificed by euthanasia, and necropsies were performed.
3 June 81	Female rats that survived were weighed, sacrificed by euthanasia,and necropsies were performed.

Changes to Original Objectives and Procedures

1. Analysis of the chemical (CHF1) was not performed. Chemical analyses were waived because the technical expertise was not available at LAIR at the time of the study.

2. During dosing, animals D8100182 (group 3), D8100134 (group 4), and D8100166 (group 4) did not receive their full dose; consequently, they were not included in summarizing the results. Animal D8100187 (group 5) died from an injury (perforated esophagus) received during dosing and was not used in summarizing the results.

3. Animals were weighed both on 7 May 1981 and 8 May 1981, a change in protocol, because of problems with getting the weights on the database for the TOXSYS® system which was being tested for use with these types of studies.

4. On 18 May 1981 the humidity in the animal room was about 80% for approximately 20 hours. On 25 May 1981 the humidity increased up to 90% several times over a 16 hour period. Otherwise, it remained within the range specified under Environmental Conditions.

5. Separate control groups for isopropanol and Dow Corning 200 Fluid were not tested as the formulation was requested to be treated as a individual unit. Data related to isopropanol and Dow Corning 200 Fluid toxicity are documented in the open scientific literature.

RESULTS

Mortality

Table 1 lists the compound related deaths by group.

TABLE 1
Compound Related Deaths by Group

Group	Dose Level	Sex	Compound Related Death/ Number in Group
1	Vehicle Control	Male	0/10
		Female	0/10
2	2000 ul/kg	Male	1/10
		Female	4/10
3	2515 ul/kg	Male	0/10
		Female	5/9[a]
4	3162 ul/kg	Male	1/8[a]
		Female	5/10
5	3976 ul/kg	Male	4/10
		Female	7/9[b]
6	5000 ul/kg	Male	6/10
		Female	10/10

[a] Animals eliminated because of misdosing, one rat in Group 3 and two in Group 4.

[b] Animal eliminated because of death from a puncture wound received during dosing.

Time of death after dosing was recorded on the data sheets and graphed by sex at the end of the study (Figure 1).

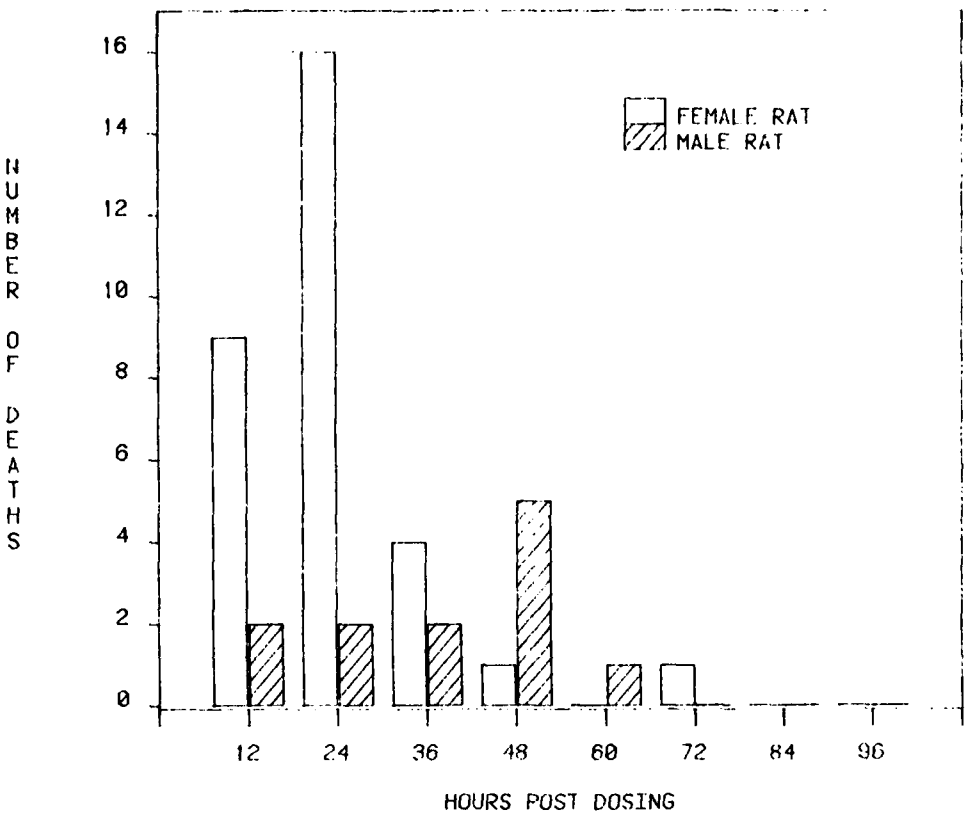


Figure 1: LD_{50} for CHF 1 (#81010), Time of Death - Male and Female Rats.

Lethal Dose Calculations

Lethal dose (LD) values calculated by probit analysis for CHF1 are given below for males (Table 2) and females (Table 3).

TABLE 2
Lethal Dose (LD) Levels of CHF1 in Male Rats

Percent Population	Lethal Dose (ul/kg)	95% Confidence Interval (ul/kg)
LD_1	1641	732 - 3678
LD_{50}	4632	3374 - 6359
LD_{95}	9647	3590 - 25930

TABLE 3
Lethal Dose (LD) Levels of CHF1 in Female Rats

Percent Population	Lethal Dose (ul/kg)	95% Confidence Interval (ul/kg)
LD_1	754	198 - 2878
LD_{50}	2495	1905 - 3268
LD_{95}	5815	2932 - 11530

Figure 2 is a graphic representation of the probit analysis derived response curves for males and females. In Figures 3 and 4 the response curves for males and females were graphed separately with their 95% confidence intervals.

The statistician's statement appears in Appendix A-1.

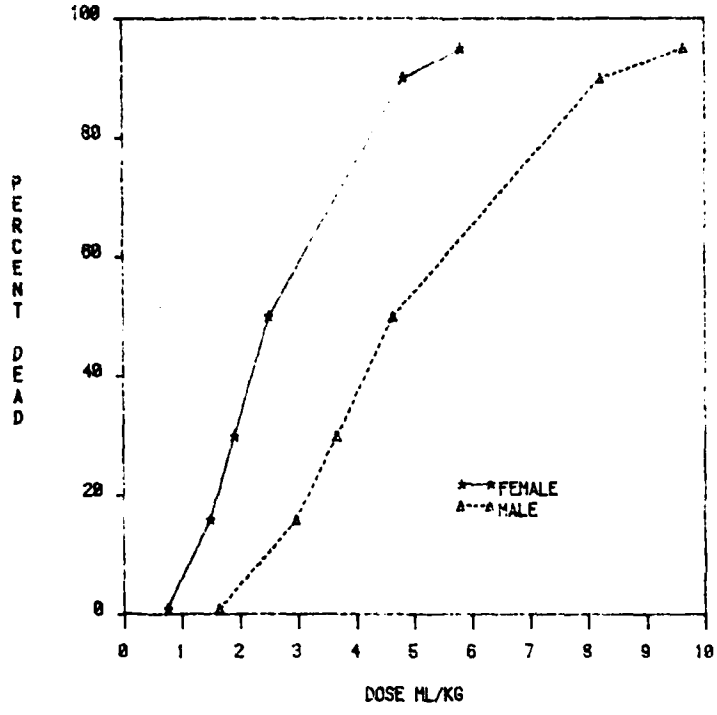


FIGURE 2: LD_{50} for CHF 1 (#81010), Probit Analysis Derived Dose Response Curve - Male and Female Rats.

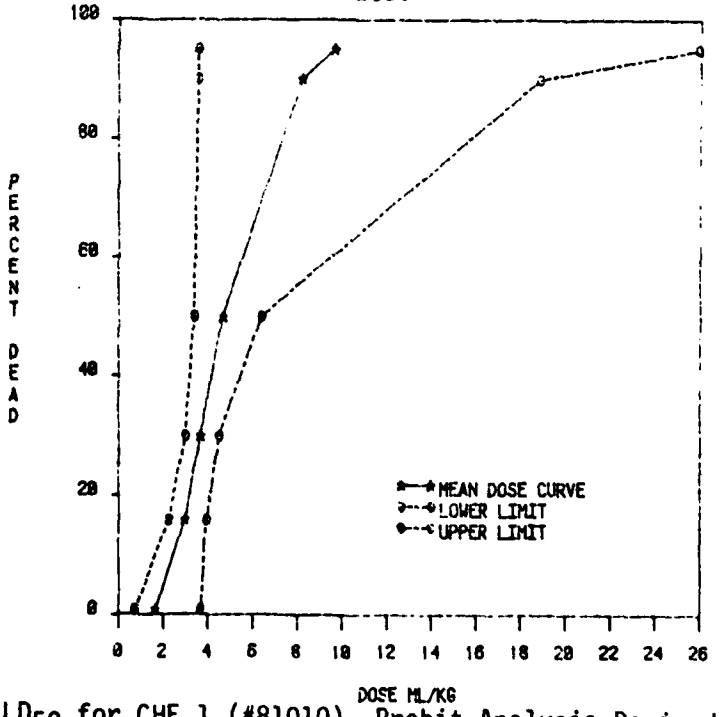


FIGURE 3: LD_{50} for CHF 1 (#81010), Probit Analysis Derived Dose Response Curve - Male Rats.

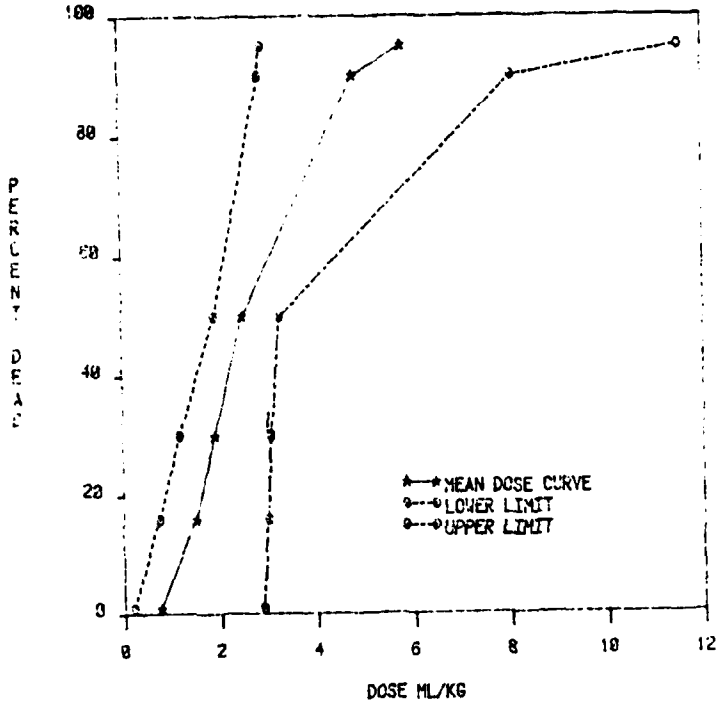


FIGURE 4: LD_{50} for CHF1 (#81010), Probit Analysis Derived Dose Response Curve - Female Rats.

Clinical Observations

Twice a day animals were observed in undisturbed cages, outside of cages and after placement in cages. Observation of clinical signs is a subjective measurement of toxicity due to variation in individuals' judgement. Furthermore, observations were only done at 12-hr intervals; consequently, they may not include all clinical signs exhibited by each animal. The purpose of recording the clinical signs was only to gain more information on the toxic effects of the formulation for use in future studies; therefore, only clinical signs of high frequency (greater than ten percent of the dosed animals per sex) will be discussed. While a quantitative summary of the incidence of clinical signs was done, statistical analysis was not done, nor are specific numbers discussed to avoid placing any more importance on them than is warranted.

In this study, a wider variety of clinical signs was seen in the male rats than the female rats. Furthermore, the incidence of a number signs was higher in the males versus the females. The signs that occurred more frequently in the males also occurred more often in the lower dose groups, regardless of sex. These findings are not surprising because rats often died before clinical signs could be observed. Therefore, the differences between sexes in the incidence of clinical signs is probably the result of differences in their survival rate and not their symptomology. The clinical signs that had a higher frequency in males include increased respiratory rate, sound production, rough coat, piloerection, decreased reflex response (particularly the righting reflex, pinch reflex and grip strength), humpback, inactivity, sluggishness, loss of equilibrium, loss of gait, red material on the head or forelegs (presumably from harderian gland secretions) and yellow perianal material or stain.

There were several clinical signs that had a higher incidence among female rats. These clinical signs were seen primarily in moribund animals, regardless of sex. The reason these signs were higher in females is presumably due to their higher death rate. Among the signs that fall into this category are decreased respiratory rate, increased or decreased respiratory depth, ocular tearing, decreased temperature and collapse. While most animals collapsed and became comatose before dying, a few males rats surprisingly recovered after collapsing.

Gross Pathological Observations

The Pathologist's Report appears in Appendix A-2.

DISCUSSION

The calculated LD_{50} for the CHF1 formulation in male rats was 4632 ul/kg with a 95% confidence interval from 3374 to 6359 ul/kg. The LD_{50} for female rats was 2495 ul/kg and had a smaller 95% confidence interval (1905 - 3268 ul/kg). The LD_{50} for males is near the "practically nontoxic" range (5 to 15 gm/kg), however, the LD_{50} for females falls clearly within the "slightly toxic" range (0.5 to 5.0 gm/kg)(2). For this reason CHF1 is best classified as "slightly toxic." The slope of the dose response curve was greater for female rats, therefore the margin of safety is narrower for females.

Of animals that died from the chemical, all died within three days after dosing. The greatest incidence of deaths among female rats was between 12 and 24 hours after dosing. Male rats lingered longer with the highest number of deaths occurring between 36 and 48 hours after dosing. The exact cause of death is not known, but gross pathological examination revealed gross lesions in the gastrointestinal tract and lungs.

Clinical signs of toxicity included sound production, rough coat, piloerection, decreased reflexes, inactivity or sluggishness, and loss of equilibrium or gait. Changes in respiratory rate and depth, ocular tearing, decreased temperature and collapse were frequently observed in moribund animals suggesting a narcotic like effect. In general, the male rats had a wider variety and higher incidence of clinical signs. However, the incidence of signs can be misleading since animals often died before many signs were recorded. Because CHF1 was more potent for female rats, the differences in the incidence of clinical signs between sexes probably reflects the differences in the survival rate rather than any real differences in the symptoms of toxicity.

CONCLUSION

The LD_{50} for CHF1 formulation was 4632 ul/kg for male rats and 2495 ul/kg for female rats. The CHF1 formulation is considered slightly toxic.

RECOMMENDATION

The CHF1 formulation should be considered for further safety testing for eventual human use.

REFERENCES

1. FINNEY, D.J. Probit Analysis Third Edition. Chapters 3 and 4. Cambridge: Cambridge University Press, 1971

2. LOOMIS, T.A. Essentials of Toxicology, Third Edition. Philadelphia: Lea and Febiger, 1978. pp 16-23

APPENDIX

A-1, Statistical Analysis

A-2, Pathology Report

APPENDIX A-1

LAIR GLP Study 81010

Acute Oral Toxicity of Male and Female Rats - CHF1

Statistical Analysis, Summary and Interpretation

Using Animal Allocate (TOXSYS ®), ten animals were randomly assigned according to their weight to each of six male and six female groups.

A Fortran IV program on a CDC 7600/6600 computer was utilized to perform Bliss' method of probit analysis for the number of animals dead per group. The program utilized the percentage kills to determine the weighted regression line of the mortality probit on the log-dose, which results in the formula for males and females, respectively:

$$Y = -13.92 + 5.16\ X$$

and $$Y = -10.20 + 4.47\ X$$

Where Y is the probit and X is the logarithm of the dose, A x^2 statistic was calculated to test the acceptable fit of each line at the .05 significance level. The male and female probits were then converted back to percentages and the LD_1, LD_{50} and LD_{90} were determined along with their 95% confidence limits to be as stated in the text.

Virginia L. Gildengorin
22 Oct 1981

VIRGINIA L. GILDENGORIN, PhD
Statistician
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

APPENDIX A-2

Gross Pathology Summary and Interpretation GLP Study 81010 LD_{50} 50% DEET + 25% Dow-Corning 200 Fluid in Isopropanol, Male Sprague Dawley Rats

Deaths attributable to toxic effects of the compound occurred in all but one of the dosage groups. The number of animals that died in each dosage group were as follows: Group 1 (controls) 0/10*; Group 2 (2000 µl/kg) 1/10; Group 3 (2515 µl/kg) 0/10; Group 4 (3162 µl/kg) 3/10; Group 5 (3976 µl/kg) 410; Group 6 (5000 µl/kg) 6/10. All deaths occurred between 8 hours, 14 minutes and 80 hours after administration of the compound by gastric intubation. Gross lesions attributable to the test material were seen in the stomachs and intestines of all animals that died. Gastric contents of these animals was usually yellow, oily, or "cheesy" in consistency Contents of the small intestine were usually either red or black, probably indicating the presence of blood, and gelatinous or mucoid in consistency. Many of these changes in gastrointestinal contents were probably altered or exaggerated by autolysis.

Gross changes were detected in lungs of animals that died in all groups. Lung changes when present consisted of congestion and edema or hemorrhage. Distribution of these lesions by dosage group was as follows:

Group 1 Control	Group 2 2000 µl/kg	Group 3 2515 µl/kg	Group 4 3162 µl/kg	Group 5 3976 µl/kg	Group 6 5000 µl/kg
Congestion with edema:					
0/10	1/10	1/10	1/10	3/10	3/10
Hemorrhage:					
0/10	1/10	1/10	1/10	2/10	1/10

Pulmonary hemorrhage, congestion and edema in these cases may have been agonal changes and accentuated by autolysis. However, the possibility that these changes could have resulted from pulmonary excretion of the test compound cannot be eliminated. One animal in the control group had an irregular raised focal grayish brown lesions that measure 6 mm x 5 mm. This was probably a focal area of inflammation, the cause of which was undetermined.

Lesions were observed in the urinary system of several animals. One animal in group 5 and one in group 6 had moderate hydronephrosis. Another animal in group 6 and one in group 4 had gray streaks in the cortical parenchyma which were probably incidental and unrelated to

*Number of rats affected/number of rats in the group.

the test material. Two animals that died had bluish green urine in their bladders. This is probably an autolytic change that may have been due to bacterial breakdown of metabolites of the test material.

Paul W. Mellick 12OCT81

PAUL W. MELLICK, DVM, PhD
Diplomate, ACVP
LTC, VC, USA

APPENDIX A-2 (cont.)

Gross Pathology Summary and Interpretation GLP Study 81010, LD_{50} 50% DEET + 25% Dow-Corning 200 Fluid in Isopropanol Female Sprague Dawley Rats

Deaths attributed to toxic effects of the test compound occurred in all groups except controls. Number of animals that died by dosage group were as follows: Group 2 (2000 µl/kg) - 4/10; Group 3 (2515 µl/kg) 6/10; Group 4 (3162 µl/kg) 5/10; Group 5 (3976 µl/kg) 8/10; and Group 6 (5000 µl/kg) 10/10*. All deaths occurred between 8 hours 9 minutes and 69 hours after administration of the compound by gastric intubation with one exception. One animal died 40 minutes after gastric intubation as a result of esophageal perforation and thoracic hemorrhage. This animal was in group 5 (3976 µl/kg). Gross lesions attributable to the test material were seen in the stomach and intestines of all animals that died. Gastric changes consisted of distension, petechial hemorrhage of the mucosa and/or contents consisting of yellow oily material mixed with mucus. Gross changes in the intestines included distension with red gelatinous material and/or yellow or green oily material in the lumens that were mixed with mucus. Some of these changes may have been exaggerated by autolysis.

Gross changes were detected in lungs of animals that died in all groups. Lung changes when present consisted of congestion and edema or hemorrhage. Distribution of these lesions by dosage group is as follows:

Group 1 (control)	Group 2 2000 µl/kg	Group 3 2515 µl/kg	Group 4 3162 µl/kg	Group 5 3976 µl/kg	Group 6 5000 µl/kg
Congestion with edema:					
0/10	1/10	2/10	0/10	4/10	4/10
Hemorrhage:					
0/10	1/10	0/10	2/10	3/10	3/10

Pulmonary hemorrhage, congestion and edema in these cases may have been due to agonal changes and accentuated by autolysis. However, the possibility that these changes could have resulted from pulmonary excretion of the test compound or its metabolites cannot be eliminated.

Thymic hemorrhages were present in one rat in group 3, two in group 4, one in group 5, and three in group 6. These changes are frequently observed in rats that have been dead for several hours and are usually considered to be a post mortem change.

*Number of rats affected/number of rats in the group.

Unilateral hydronephrosis was observed in one rat in group 2 and two animals in group 3. This is a common lesion in Sprague Dawley rats. These lesions were considered to be incidental findings unrelated to administration of the test compound.

Paul W. Mellick 22 Oct 81

PAUL W. MELLICK, DVM, PhD
Diplomate, ACVP
LTC, VC, USA

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